

SEQUENCE LISTING

<110> Xu, Shuang-yong
Kobbe, Daniela
Zhu, Zhenyu
Samuelson, James

<120> Methods for Altering the Cleavage Specificity of a Type II
Restriction Endonuclease

<130> NEB-183-CIP

<150> 10/150,028

<151> 2002-05-17

<150> 09/693,146

<151> 2000-07-02

<160> 26

<170> PatentIn version 3.2

<210> 1

<211> 1650

<212> DNA

<213> Bacillus pumilus

<220>

<221> CDS

<222> (1)..(1650)

<400> 1

atg	aat	caa	tta	att	gaa	aat	gtt	aat	cta	caa	aaa	tta	agg	ggt	ggg	48
Met	Asn	Gln	Leu	Ile	Glu	Asn	Val	Asn	Leu	Gln	Lys	Leu	Arg	Gly	Gly	
1				5					10					15		

tat	tac	acc	cct	aaa	gtt	att	gct	gac	ttt	tta	tgt	caa	tgg	agt	att	96
Tyr	Tyr	Thr	Pro	Lys	Val	Ile	Ala	Asp	Phe	Leu	Cys	Gln	Trp	Ser	Ile	
			20					25					30			

caa	gat	gac	aca	aag	agt	gta	ctt	gaa	ccc	agt	tgt	gga	gat	ggt	aat	144
Gln	Asp	Asp	Thr	Lys	Ser	Val	Leu	Glu	Pro	Ser	Cys	Gly	Asp	Gly	Asn	
		35					40					45				

ttt	att	gaa	tcg	gca	ata	ctt	agg	ttc	aaa	gaa	ctt	agt	ata	gat	aat	192
Phe	Ile	Glu	Ser	Ala	Ile	Leu	Arg	Phe	Lys	Glu	Leu	Ser	Ile	Asp	Asn	
	50					55					60					

gaa	caa	ctt	aaa	gga	aga	att	aca	gga	gta	gag	cta	att	gaa	gaa	gaa	240
Glu	Gln	Leu	Lys	Gly	Arg	Ile	Thr	Gly	Val	Glu	Leu	Ile	Glu	Glu	Glu	
65					70					75					80	

gct	ttg	aaa	gtt	caa	aat	cga	gca	aat	gag	ttg	ggg	gtt	gat	aaa	aac	288
Ala	Leu	Lys	Val	Gln	Asn	Arg	Ala	Asn	Glu	Leu	Gly	Val	Asp	Lys	Asn	
				85					90					95		

tca	ata	gta	aat	agt	gac	ttc	ttt	caa	ttt	gta	aaa	gat	aat	aag	aat	336
Ser	Ile	Val	Asn	Ser	Asp	Phe	Phe	Gln	Phe	Val	Lys	Asp	Asn	Lys	Asn	
			100					105					110			

aaa	aaa	ttt	gat	act	att	att	ggt	aat	cca	cca	ttc	ata	aga	tac	caa	384
Lys	Lys	Phe	Asp	Thr	Ile	Ile	Gly	Asn	Pro	Pro	Phe	Ile	Arg	Tyr	Gln	

neb-183-cip.txt

115		120		125	
aac ttt cct gaa gag cat cgt agt ata gcc atg gaa atg atg gag gaa	432	Asn Phe Pro Glu Glu His Arg Ser Ile Ala Met Glu Met Met Glu Glu	130	135	140
cta ggt tta aaa cct aat aaa ctt aca aat atc tgg gtt cca ttt cta	480	Leu Gly Leu Lys Pro Asn Lys Leu Thr Asn Ile Trp Val Pro Phe Leu	145	150	155
gtg gta tct gct aca tta ctt aat gaa caa gga aag atg gct atg gtt	528	Val Val Ser Ala Thr Leu Leu Asn Glu Gln Gly Lys Met Ala Met Val	165	170	175
ata ccg gct gaa tta ttt cag gta aag tat gca gca gaa aca aga att	576	Ile Pro Ala Glu Leu Phe Gln Val Lys Tyr Ala Ala Glu Thr Arg Ile	180	185	190
ttt tta tca aag ttt ttc gat cgt atc act ata att aca ttt gaa aaa	624	Phe Leu Ser Lys Phe Phe Asp Arg Ile Thr Ile Ile Thr Phe Glu Lys	195	200	205
ctt gtt ttt gaa aat atc caa cag gaa gtt ata cta ctt ctt tgt gaa	672	Leu Val Phe Glu Asn Ile Gln Gln Glu Val Ile Leu Leu Leu Cys Glu	210	215	220
aag aaa gtt aat aaa ggt aaa gga att cgg gtt att gaa tgc gag aac	720	Lys Lys Val Asn Lys Gly Lys Gly Ile Arg Val Ile Glu Cys Glu Asn	225	230	235
tta gat gga tta aat tcc att gat ttt gta gct ata aat ggt tca aat	768	Leu Asp Gly Leu Asn Ser Ile Asp Phe Val Ala Ile Asn Gly Ser Asn	245	250	255
gtt aaa cct att gaa cac cgt act gaa aag tgg aca aag tat ttc tta	816	Val Lys Pro Ile Glu His Arg Thr Glu Lys Trp Thr Lys Tyr Phe Leu	260	265	270
aac gaa gat gaa ata ctt ctt tta cag agt tta aag gaa gac aaa cgc	864	Asn Glu Asp Glu Ile Leu Leu Leu Gln Ser Leu Lys Glu Asp Lys Arg	275	280	285
gtt aaa aat tgt aat gac tat ttt aag aca gaa gtt ggc tta gtt act	912	Val Lys Asn Cys Asn Asp Tyr Phe Lys Thr Glu Val Gly Leu Val Thr	290	295	300
gga cga aac gaa ttc ttt atg atg aaa gaa aac caa gta aaa gaa tgg	960	Gly Arg Asn Glu Phe Phe Met Met Lys Glu Asn Gln Val Lys Glu Trp	305	310	315
aat cta gaa gaa tat aca ata cct gtt aca ggt agg tcc aat cag tta	1008	Asn Leu Glu Glu Tyr Thr Ile Pro Val Thr Gly Arg Ser Asn Gln Leu	325	330	335
aaa ggt ata aca ttt aca gaa aat gat ttt cat gaa aat tca atg gaa	1056	Lys Gly Ile Thr Phe Thr Glu Asn Asp Phe His Glu Asn Ser Met Glu	340	345	350
caa aag gca att cac cta ttt ttg cca cca gat gaa gat ttt gaa aag	1104	Gln Lys Ala Ile His Leu Phe Leu Pro Pro Asp Glu Asp Phe Glu Lys	355	360	365

neb-183-cip.txt

tta ccg att gag tgt caa aat tat atc aag tat ggg gaa gaa aaa ggc Leu Pro Ile Glu Cys Gln Asn Tyr Ile Lys Tyr Gly Glu Glu Lys Gly	1152
370 375 380	
ttc cat caa ggc tat aaa acc aga att aga aaa cgt tgg tat ata act Phe His Gln Gly Tyr Lys Thr Arg Ile Arg Lys Arg Trp Tyr Ile Thr	1200
385 390 395 400	
cca tct aga tgg gtt cca gat gct ttt gct tta aga cag gtt gat ggc Pro Ser Arg Trp Val Pro Asp Ala Phe Ala Leu Arg Gln Val Asp Gly	1248
405 410 415	
tat cca aaa cta att tta aat gaa acc gac gct tct tct act gat aca Tyr Pro Lys Leu Ile Leu Asn Glu Thr Asp Ala Ser Ser Thr Asp Thr	1296
420 425 430	
att cat agg gtt aga ttt aaa gaa ggt ata aat gaa aag tta gcc gta Ile His Arg Val Arg Phe Lys Glu Gly Ile Asn Glu Lys Leu Ala Val	1344
435 440 445	
gtt tca ttt ttg aac tca ctc act ttt gca tct tca gaa ata acg ggg Val Ser Phe Leu Asn Ser Leu Thr Phe Ala Ser Ser Glu Ile Thr Gly	1392
450 455 460	
aga agt tat ggt ggt ggt gtt atg aca ttc gaa cca act gaa att gga Arg Ser Tyr Gly Gly Gly Val Met Thr Phe Glu Pro Thr Glu Ile Gly	1440
465 470 475 480	
gaa atc cta ata cct tcc ttt gat aac tta tcc att gat ttt gat aaa Glu Ile Leu Ile Pro Ser Phe Asp Asn Leu Ser Ile Asp Phe Asp Lys	1488
485 490 495	
att gat gcc tta att cga gaa aag gag att gaa aaa gtc ctt gat att Ile Asp Ala Leu Ile Arg Glu Lys Glu Ile Glu Lys Val Leu Asp Ile	1536
500 505 510	
gtt gat gaa gct tta ctt ata aaa tat cat ggg ttt agt gag aaa gaa Val Asp Glu Ala Leu Leu Ile Lys Tyr His Gly Phe Ser Glu Lys Glu	1584
515 520 525	
gta aaa cag ctt cga ggg ata tgg aag aaa ctt tct cag aga aga aac Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn	1632
530 535 540	
aat aga acg aag aaa taa Asn Arg Thr Lys Lys	1650
545 550	

<210> 2

<211> 549

<212> PRT

<213> Bacillus pumilus

<400> 2

Met Asn Gln Leu Ile Glu Asn Val Asn Leu Gln Lys Leu Arg Gly Gly
1 5 10 15

Tyr Tyr Thr Pro Lys Val Ile Ala Asp Phe Leu Cys Gln Trp Ser Ile
20 25 30

Gln Asp Asp Thr Lys Ser Val Leu Glu Pro Ser Cys Gly Asp Gly Asn

35 40 45
 Phe Ile Glu Ser Ala Ile Leu Arg Phe Lys Glu Leu Ser Ile Asp Asn
 50 55 60
 Glu Gln Leu Lys Gly Arg Ile Thr Gly Val Glu Leu Ile Glu Glu Glu
 65 70 75 80
 Ala Leu Lys Val Gln Asn Arg Ala Asn Glu Leu Gly Val Asp Lys Asn
 85 90 95
 Ser Ile Val Asn Ser Asp Phe Phe Gln Phe Val Lys Asp Asn Lys Asn
 100 105 110
 Lys Lys Phe Asp Thr Ile Ile Gly Asn Pro Pro Phe Ile Arg Tyr Gln
 115 120 125
 Asn Phe Pro Glu Glu His Arg Ser Ile Ala Met Glu Met Met Glu Glu
 130 135 140
 Leu Gly Leu Lys Pro Asn Lys Leu Thr Asn Ile Trp Val Pro Phe Leu
 145 150 155 160
 Val Val Ser Ala Thr Leu Leu Asn Glu Gln Gly Lys Met Ala Met Val
 165 170 175
 Ile Pro Ala Glu Leu Phe Gln Val Lys Tyr Ala Ala Glu Thr Arg Ile
 180 185 190
 Phe Leu Ser Lys Phe Phe Asp Arg Ile Thr Ile Ile Thr Phe Glu Lys
 195 200 205
 Leu Val Phe Glu Asn Ile Gln Gln Glu Val Ile Leu Leu Leu Cys Glu
 210 215 220
 Lys Lys Val Asn Lys Gly Lys Gly Ile Arg Val Ile Glu Cys Glu Asn
 225 230 235 240
 Leu Asp Gly Leu Asn Ser Ile Asp Phe Val Ala Ile Asn Gly Ser Asn
 245 250 255
 Val Lys Pro Ile Glu His Arg Thr Glu Lys Trp Thr Lys Tyr Phe Leu
 260 265 270
 Asn Glu Asp Glu Ile Leu Leu Leu Gln Ser Leu Lys Glu Asp Lys Arg
 275 280 285
 Val Lys Asn Cys Asn Asp Tyr Phe Lys Thr Glu Val Gly Leu Val Thr
 290 295 300
 Gly Arg Asn Glu Phe Phe Met Met Lys Glu Asn Gln Val Lys Glu Trp
 305 310 315 320
 Asn Leu Glu Glu Tyr Thr Ile Pro Val Thr Gly Arg Ser Asn Gln Leu
 325 330 335
 Lys Gly Ile Thr Phe Thr Glu Asn Asp Phe His Glu Asn Ser Met Glu
 340 345 350
 Gln Lys Ala Ile His Leu Phe Leu Pro Pro Asp Glu Asp Phe Glu Lys
 355 360 365

neb-183-cip.txt

Leu Pro Ile Glu Cys Gln Asn Tyr Ile Lys Tyr Gly Glu Glu Lys Gly
 370 375 380
 Phe His Gln Gly Tyr Lys Thr Arg Ile Arg Lys Arg Trp Tyr Ile Thr
 385 390 395 400
 Pro Ser Arg Trp Val Pro Asp Ala Phe Ala Leu Arg Gln Val Asp Gly
 405 410 415
 Tyr Pro Lys Leu Ile Leu Asn Glu Thr Asp Ala Ser Ser Thr Asp Thr
 420 425 430
 Ile His Arg Val Arg Phe Lys Glu Gly Ile Asn Glu Lys Leu Ala Val
 435 440 445
 Val Ser Phe Leu Asn Ser Leu Thr Phe Ala Ser Ser Glu Ile Thr Gly
 450 455 460
 Arg Ser Tyr Gly Gly Gly Val Met Thr Phe Glu Pro Thr Glu Ile Gly
 465 470 475 480
 Glu Ile Leu Ile Pro Ser Phe Asp Asn Leu Ser Ile Asp Phe Asp Lys
 485 490 495
 Ile Asp Ala Leu Ile Arg Glu Lys Glu Ile Glu Lys Val Leu Asp Ile
 500 505 510
 Val Asp Glu Ala Leu Leu Ile Lys Tyr His Gly Phe Ser Glu Lys Glu
 515 520 525

Val Lys Gln Leu Arg Gly Ile Trp Lys Lys Leu Ser Gln Arg Arg Asn
 530 535 540
 Asn Arg Thr Lys Lys
 545

<210> 3
 <211> 3030
 <212> DNA
 <213> Bacillus pumilus

<220>
 <221> CDS
 <222> (1)..(3030)

<400> 3
 atg cat ata agt gag tta gta gat aaa tac aaa gcg cat aga agt act 48
 Met His Ile Ser Glu Leu Val Asp Lys Tyr Lys Ala His Arg Ser Thr
 1 5 10 15
 ttt tta aaa cca act tat aat gaa act caa cta agg aat gat ttt ata 96
 Phe Leu Lys Pro Thr Tyr Asn Glu Thr Gln Leu Arg Asn Asp Phe Ile
 20 25 30
 gac cca ctt cta aaa tct tta gga tgg gat gtt gat aat acc aaa gga 144
 Asp Pro Leu Leu Lys Ser Leu Gly Trp Asp Val Asp Asn Thr Lys Gly
 35 40 45
 aaa aca cat att cta aga gat gtc att caa gaa gaa tac ata gaa ata 192
 Page 5

neb-183-cip.txt

Lys	Thr	His	Ile	Leu	Arg	Asp	Val	Ile	Gln	Glu	Glu	Tyr	Ile	Glu	Ile		
50						55					60						
aaa	gat	gag	gag	aca	aag	aaa	aat	cca	gat	tat	aca	ctt	cgt	ata	aac	240	
Lys	Asp	Glu	Glu	Thr	Lys	Lys	Asn	Pro	Asp	Tyr	Thr	Leu	Arg	Ile	Asn	80	
65					70					75							
ggt	acg	aga	aag	ctg	ttt	gta	gag	ggt	aag	aaa	ccg	tct	ttt	aat	att	288	
Gly	Thr	Arg	Lys	Leu	Phe	Val	Glu	Val	Lys	Lys	Pro	Ser	Phe	Asn	Ile	95	
				85					90								
ttg	aaa	tca	gct	aaa	gca	gcc	ttc	caa	aca	aga	aga	tat	ggt	tgg	agt	336	
Leu	Lys	Ser	Ala	Lys	Ala	Ala	Phe	Gln	Thr	Arg	Arg	Tyr	Gly	Trp	Ser	110	
			100					105									
gct	aac	ctt	ggt	att	tca	gta	ctt	aca	aat	ttc	gag	cat	cta	gtt	att	384	
Ala	Asn	Leu	Gly	Ile	Ser	Val	Leu	Thr	Asn	Phe	Glu	His	Leu	Val	Ile	125	
		115					120										
tat	gat	tgt	aga	tat	acg	cct	gac	aaa	tcc	gac	aat	gaa	cat	att	gct	432	
Tyr	Asp	Cys	Arg	Tyr	Thr	Pro	Asp	Lys	Ser	Asp	Asn	Glu	His	Ile	Ala	140	
	130					135											
aga	tat	aaa	gtt	ttc	tct	tac	gag	gaa	tat	gaa	gaa	gca	ttt	gat	gaa	480	
Arg	Tyr	Lys	Val	Phe	Ser	Tyr	Glu	Glu	Tyr	Glu	Glu	Ala	Phe	Asp	Glu	160	
					150					155							
ata	aag	gat	ata	att	tca	tat	gag	tca	gcc	aac	tca	ggt	gct	ctg	gac	528	
Ile	Lys	Asp	Ile	Ile	Ser	Tyr	Glu	Ser	Ala	Asn	Ser	Gly	Ala	Leu	Asp	175	
				165					170								
gaa	atg	ttt	gat	gta	aat	aca	aga	gtt	ggt	gaa	acg	ttt	gac	gag	tat	576	
Glu	Met	Phe	Asp	Val	Asn	Thr	Arg	Val	Gly	Glu	Thr	Phe	Asp	Glu	Tyr	180	
			180					185					190				
ttt	tta	cag	caa	att	gag	aat	tgg	cgc	gaa	aag	cta	gct	aaa	act	gca	624	
Phe	Leu	Gln	Gln	Ile	Glu	Asn	Trp	Arg	Glu	Lys	Leu	Ala	Lys	Thr	Ala	205	
		195					200										
att	aaa	aat	aac	acc	gaa	tta	ggt	gaa	gag	gac	gtc	aat	ttt	att	gtc	672	
Ile	Lys	Asn	Asn	Thr	Glu	Leu	Gly	Glu	Glu	Asp	Val	Asn	Phe	Ile	Val	220	
	210					215											
caa	aga	cta	tta	aac	aga	att	att	ttt	ctt	aga	gtt	tgt	gaa	gat	aga	720	
Gln	Arg	Leu	Leu	Asn	Arg	Ile	Ile	Phe	Leu	Arg	Val	Cys	Glu	Asp	Arg	235	
					230					235					240		
acc	att	gaa	aaa	tat	gaa	aca	att	aaa	agt	ata	aaa	aac	tat	gag	gaa	768	
Thr	Ile	Glu	Lys	Tyr	Glu	Thr	Ile	Lys	Ser	Ile	Lys	Asn	Tyr	Glu	Glu	255	
				245					250								
tta	aaa	gat	ctg	ttt	caa	aag	tct	gat	agg	aaa	ttt	aat	tca	ggt	ctc	816	
Leu	Lys	Asp	Leu	Phe	Gln	Lys	Ser	Asp	Arg	Lys	Phe	Asn	Ser	Gly	Leu	260	
								265					270				
ttt	gac	ttc	ata	gat	gat	acg	ctc	ttg	ctt	gag	gtt	gaa	att	gat	tcg	864	
Phe	Asp	Phe	Ile	Asp	Asp	Thr	Leu	Leu	Leu	Glu	Val	Glu	Ile	Asp	Ser	285	
		275					280										
aat	gta	ttg	ata	gaa	att	ttt	agt	gat	tta	tat	ttc	cca	caa	agc	cca	912	
Asn	Val	Leu	Ile	Glu	Ile	Phe	Ser	Asp	Leu	Tyr	Phe	Pro	Gln	Ser	Pro	290	
						295					300						

neb-183-cip.txt

tat Tyr 305	gat Asp	ttt Phe	tct Ser	gtt Val	gtc Val 310	gat Asp	cca Pro	aca Thr	ata Ile	tta Leu 315	agc Ser	cag Gln	ata Ile	tat Tyr	gaa Glu 320	960
cgt Arg	ttt Phe	cta Leu	ggg Gly	caa Gln 325	gaa Glu	ata Ile	att Ile	ata Ile	gag Glu 330	tca Ser	ggg Gly	ggg Gly	aca Thr	ttt Phe 335	cac His	1008
att Ile	acg Thr	gag Glu	tca Ser 340	cca Pro	gaa Glu	gtt Val	gcg Ala	gcg Ala 345	tcc Ser	aat Asn	ggg Gly	gtt Val 350	gtt Val 350	cca Pro	act Thr	1056
cca Pro	aaa Lys	att Ile 355	atc Ile	gtc Val	gaa Glu	cag Gln	ata Ile 360	gtg Val	aaa Lys	gac Asp	act Thr	tta Leu 365	acg Thr	ccc Pro	ctt Leu	1104
acg Thr	gaa Glu 370	ggc Gly	aaa Lys	aaa Lys	ttt Phe	aat Asn 375	gag Glu	cta Leu	tgt Cys	aac Asn	tta Leu 380	aaa Lys	ata Ile	gca Ala	gat Asp	1152
ata Ile 385	tgt Cys	tgt Cys	gga Gly	tca Ser	gga Gly 390	act Thr	ttc Phe	cta Leu	att Ile	tca Ser 395	agt Ser	tat Tyr	gac Asp	ttt Phe	cta Leu 400	1200
gta Val	gag Glu	aaa Lys	gta Val	atg Met 405	gaa Glu	aag Lys	ata Ile	ata Ile	gaa Glu 410	gag Glu	aac Asn	atc Ile	gat Asp	gat Asp 415	tca Ser	1248
gat Asp	tta Leu	gta Val	tat Tyr 420	gaa Glu	act Thr	gaa Glu	gaa Glu	ggg Gly 425	cta Leu	att Ile	ttg Leu	aca Thr	ctt Leu 430	aaa Lys	gca Ala	1296
aaa Lys	aga Arg	aat Asn 435	atc Ile	ttg Leu	gag Glu	aat Asn	aat Asn 440	ttg Leu	ttt Phe	ggg Gly	gtt Val	gat Asp 445	gtt Val	aat Asn	cca Pro	1344
tac Tyr	gct Ala 450	gtt Val	gaa Glu	gta Val	gct Ala 455	gag Glu	ttc Phe	agt Ser	tta Leu	tta Leu	tta Leu 460	aag Lys	cta Leu	tta Leu	gaa Glu	1392
ggg Gly 465	gag Glu	aat Asn	gag Glu	gca Ala	tcg Ser 470	gtt Val	aat Asn	aat Asn	ttc Phe	att Ile 475	cac His	gag Glu	cat His	gag Glu	gat Asp 480	1440
aaa Lys	ata Ile	tta Leu	ccg Pro	gat Asp 485	tta Leu	aca Thr	tct Ser	att Ile	att Ile 490	aaa Lys	tgt Cys	gga Gly	aac Asn	agc Ser 495	tta Leu	1488
gta Val	gat Asp	aat Asn	aag Lys 500	ttt Phe	ttt Phe	gaa Glu	ttc Phe	atg Met 505	cca Pro	gaa Glu	tcg Ser	tta Leu	gag Glu 510	gac Asp	gat Asp	1536
gaa Glu	atc Ile	tta Leu 515	ttt Phe	aag Lys	gct Ala	aat Asn	cca Pro 520	ttt Phe	gaa Glu	tgg Trp	gaa Glu	gag Glu 525	gag Glu	ttt Phe	cca Pro	1584
gat Asp	att Ile 530	atg Met	gca Ala	aat Asn	ggg Gly	ggc Gly 535	ttt Phe	gat Asp	gct Ala	att Ile	ata Ile 540	gga Gly	aat Asn	cca Pro	cct Pro	1632
tat Tyr	gtt Val	cga Arg	ata Ile	cag Gln	aac Asn	atg Met	aaa Lys	aaa Lys	tat Tyr	agt Ser	cct Pro	gag Glu	gaa Glu	att Ile	gaa Glu	1680

neb-183-cip.txt

545		550		555		560										
tat	tat	caa	tca	aaa	gac	tct	gaa	tat	act	ggt	gca	aaa	aaa	gaa	aca	1728
Tyr	Tyr	Gln	Ser	Lys	Asp	Ser	Glu	Tyr	Thr	Val	Ala	Lys	Lys	Glu	Thr	
				565					570					575		
ggt	gac	aag	tat	ttt	tta	ttt	att	gag	aga	gca	tta	ata	tta	ctc	aat	1776
Val	Asp	Lys	Tyr	Phe	Leu	Phe	Ile	Glu	Arg	Ala	Leu	Ile	Leu	Leu	Asn	
			580					585					590			
cct	act	ggg	ctg	ttg	ggt	tat	ata	ata	ccg	cat	aaa	ttc	ttt	att	aca	1824
Pro	Thr	Gly	Leu	Leu	Gly	Tyr	Ile	Ile	Pro	His	Lys	Phe	Phe	Ile	Thr	
		595					600					605				
aaa	ggt	ggt	aag	gaa	cta	aga	aag	ttc	ata	gct	gaa	aaa	cat	caa	ata	1872
Lys	Gly	Gly	Lys	Glu	Leu	Arg	Lys	Phe	Ile	Ala	Glu	Lys	His	Gln	Ile	
	610					615					620					
tca	aaa	att	ata	aat	ttt	ggt	ggt	aca	cag	gtc	ttt	cca	gga	aga	gcg	1920
Ser	Lys	Ile	Ile	Asn	Phe	Gly	Val	Thr	Gln	Val	Phe	Pro	Gly	Arg	Ala	
					630					635					640	
aca	tat	acg	gct	att	tta	att	atc	caa	gca	aat	aaa	atg	gca	cag	ttc	1968
Thr	Tyr	Thr	Ala	Ile	Leu	Ile	Ile	Gln	Ala	Asn	Lys	Met	Ala	Gln	Phe	
				645					650					655		
aag	tat	aag	aaa	gta	agt	aat	ata	tca	gca	gaa	acc	cta	gat	tct	gaa	2016
Lys	Tyr	Lys	Lys	Val	Ser	Asn	Ile	Ser	Ala	Glu	Thr	Leu	Asp	Ser	Glu	
			660					665					670			
gaa	aat	acg	tgt	ggt	tat	agc	tca	gaa	aag	tat	aat	tct	gac	cct	tgg	2064
Glu	Asn	Thr	Cys	Val	Tyr	Ser	Ser	Glu	Lys	Tyr	Asn	Ser	Asp	Pro	Trp	
		675					680					685				
ata	ttt	tta	tct	cct	gaa	aca	gaa	gct	ggt	ttt	act	aaa	ttt	aca	gaa	2112
Ile	Phe	Leu	Ser	Pro	Glu	Thr	Glu	Ala	Val	Phe	Thr	Lys	Phe	Thr	Glu	
	690					695					700					
gct	caa	ttt	gag	aaa	ctt	gga	gaa	atc	act	gat	ata	agt	gta	gga	cta	2160
Ala	Gln	Phe	Glu	Lys	Leu	Gly	Glu	Ile	Thr	Asp	Ile	Ser	Val	Gly	Leu	
					710					715					720	
caa	aca	agc	gct	gat	aaa	ata	tat	att	ttt	att	cct	gaa	aat	gaa	act	2208
Gln	Thr	Ser	Ala	Asp	Lys	Ile	Tyr	Ile	Phe	Ile	Pro	Glu	Asn	Glu	Thr	
				725					730					735		
tca	gat	aca	tat	ata	ttt	aat	tat	aaa	ggg	aaa	aga	tat	gaa	ata	gaa	2256
Ser	Asp	Thr	Tyr	Ile	Phe	Asn	Tyr	Lys	Gly	Lys	Arg	Tyr	Glu	Ile	Glu	
			740					745					750			
aaa	tct	ata	tgt	tgc	cca	gct	atc	tat	gac	tta	tct	ttt	ggt	tct	ttt	2304
Lys	Ser	Ile	Cys	Cys	Pro	Ala	Ile	Tyr	Asp	Leu	Ser	Phe	Gly	Ser	Phe	
		755					760					765				
gaa	agc	att	cag	gga	aat	gca	caa	atg	ata	ttc	cct	tat	gaa	atc	aga	2352
Glu	Ser	Ile	Gln	Gly	Asn	Ala	Gln	Met	Ile	Phe	Pro	Tyr	Glu	Ile	Arg	
		770				775					780					
gat	gaa	gaa	gca	tat	cta	cta	gag	gaa	gaa	acg	ctt	gaa	aat	gat	tat	2400
Asp	Glu	Glu	Ala	Tyr	Leu	Leu	Glu	Glu	Glu	Thr	Leu	Glu	Asn	Asp	Tyr	
					790					795					800	

neb-183-cip.txt

cct	ctt	gct	tgg	aat	tat	ttg	aat	gag	ttt	aaa	gaa	gct	ctt	gaa	aaa	2448
Pro	Leu	Ala	Trp	Asn	Tyr	Leu	Asn	Glu	Phe	Lys	Glu	Ala	Leu	Glu	Lys	
				805					810					815		
aga	agc	tta	caa	ggc	cgt	aat	ccg	aaa	tgg	tat	caa	tat	ggg	cgg	tcc	2496
Arg	Ser	Leu	Gln	Gly	Arg	Asn	Pro	Lys	Trp	Tyr	Gln	Tyr	Gly	Arg	Ser	
			820					825					830			
caa	agt	tta	tca	aaa	ttt	cat	gat	aaa	gaa	aaa	ctg	ata	tgg	acc	gta	2544
Gln	Ser	Leu	Ser	Lys	Phe	His	Asp	Lys	Glu	Lys	Leu	Ile	Trp	Thr	Val	
		835					840					845				
ctt	gct	acg	aaa	ccc	ccg	tat	gta	ctt	gat	agg	aat	aac	ctg	tta	ttt	2592
Leu	Ala	Thr	Lys	Pro	Pro	Tyr	Val	Leu	Asp	Arg	Asn	Asn	Leu	Leu	Phe	
	850					855					860					
act	ggg	ggg	gga	aac	gga	ccg	tat	tat	ggg	tta	att	aac	caa	tct	att	2640
Thr	Gly	Gly	Gly	Asn	Gly	Pro	Tyr	Tyr	Gly	Leu	Ile	Asn	Gln	Ser	Ile	
865					870					875					880	
tac	tct	ttg	cat	tat	ttt	tta	ggg	att	ctt	tca	cat	cct	gta	ata	gaa	2688
Tyr	Ser	Leu	His	Tyr	Phe	Leu	Gly	Ile	Leu	Ser	His	Pro	Val	Ile	Glu	
				885					890					895		
agt	atg	gta	aaa	gca	agg	gcc	agt	gaa	ttt	agg	gga	tca	tat	tat	tct	2736
Ser	Met	Val	Lys	Ala	Arg	Ala	Ser	Glu	Phe	Arg	Gly	Ser	Tyr	Tyr	Ser	
			900					905					910			
cat	gga	aaa	caa	ttt	att	gag	aaa	atc	cca	att	aga	aag	att	gat	ttt	2784
His	Gly	Lys	Gln	Phe	Ile	Glu	Lys	Ile	Pro	Ile	Arg	Lys	Ile	Asp	Phe	
		915					920					925				
gat	gat	caa	gat	gag	gta	gac	aaa	tat	aat	acg	gtg	gtc	aca	aca	gta	2832
Asp	Asp	Gln	Asp	Glu	Val	Asp	Lys	Tyr	Asn	Thr	Val	Val	Thr	Thr	Val	
	930					935					940					
gaa	aaa	tta	att	ata	act	acc	gat	aga	att	aaa	agt	gag	agc	aat	gga	2880
Glu	Lys	Leu	Ile	Ile	Thr	Thr	Asp	Arg	Ile	Lys	Ser	Glu	Ser	Asn	Gly	
945					950					955					960	
ccc	cgg	agg	aga	atg	tta	aga	aga	agg	tta	gat	gct	ttg	tct	aat	caa	2928
Pro	Arg	Arg	Arg	Met	Leu	Arg	Arg	Arg	Leu	Asp	Ala	Leu	Ser	Asn	Gln	
				965					970					975		
ctt	atc	cag	gtt	att	aat	gaa	ctt	tat	aat	atc	agt	gac	gaa	gaa	tat	2976
Leu	Ile	Gln	Val	Ile	Asn	Glu	Leu	Tyr	Asn	Ile	Ser	Asp	Glu	Glu	Tyr	
			980					985					990			
acg	aca	gtt	ttg	aat	gat	gaa	atg	ttg	aca	gcg	gcg	tta	gga	gaa	gaa	3024
Thr	Thr	Val	Leu	Asn	Asp	Glu	Met	Leu	Thr	Ala	Ala	Leu	Gly	Glu	Glu	
		995					1000					1005				
aaa	tga															3030
Lys																
	1010															

<210> 4
 <211> 1009
 <212> PRT
 <213> Bacillus pumilus

<400> 4
 Met His Ile Ser Glu Leu Val Asp Lys Tyr Lys Ala His Arg Ser Thr
 1 5 10 15
 Phe Leu Lys Pro Thr Tyr Asn Glu Thr Gln Leu Arg Asn Asp Phe Ile
 20 25 30
 Asp Pro Leu Leu Lys Ser Leu Gly Trp Asp Val Asp Asn Thr Lys Gly
 35 40 45
 Lys Thr His Ile Leu Arg Asp Val Ile Gln Glu Glu Tyr Ile Glu Ile
 50 55 60
 Lys Asp Glu Glu Thr Lys Lys Asn Pro Asp Tyr Thr Leu Arg Ile Asn
 65 70 75 80
 Gly Thr Arg Lys Leu Phe Val Glu Val Lys Lys Pro Ser Phe Asn Ile
 85 90 95
 Leu Lys Ser Ala Lys Ala Ala Phe Gln Thr Arg Arg Tyr Gly Trp Ser
 100 105 110
 Ala Asn Leu Gly Ile Ser Val Leu Thr Asn Phe Glu His Leu Val Ile
 115 120 125
 Tyr Asp Cys Arg Tyr Thr Pro Asp Lys Ser Asp Asn Glu His Ile Ala
 130 135 140
 Arg Tyr Lys Val Phe Ser Tyr Glu Glu Tyr Glu Glu Ala Phe Asp Glu
 145 150 155 160
 Ile Lys Asp Ile Ile Ser Tyr Glu Ser Ala Asn Ser Gly Ala Leu Asp
 165 170 175
 Glu Met Phe Asp Val Asn Thr Arg Val Gly Glu Thr Phe Asp Glu Tyr
 180 185 190
 Phe Leu Gln Gln Ile Glu Asn Trp Arg Glu Lys Leu Ala Lys Thr Ala
 195 200 205
 Ile Lys Asn Asn Thr Glu Leu Gly Glu Glu Asp Val Asn Phe Ile Val
 210 215 220
 Gln Arg Leu Leu Asn Arg Ile Ile Phe Leu Arg Val Cys Glu Asp Arg
 225 230 235 240
 Thr Ile Glu Lys Tyr Glu Thr Ile Lys Ser Ile Lys Asn Tyr Glu Glu
 245 250 255
 Leu Lys Asp Leu Phe Gln Lys Ser Asp Arg Lys Phe Asn Ser Gly Leu
 260 265 270
 Phe Asp Phe Ile Asp Asp Thr Leu Leu Leu Glu Val Glu Ile Asp Ser
 275 280 285

neb-183-cip.txt

Asn Val Leu Ile Glu Ile Phe Ser Asp Leu Tyr Phe Pro Gln Ser Pro
 290 295 300
 Tyr Asp Phe Ser Val Val Asp Pro Thr Ile Leu Ser Gln Ile Tyr Glu
 305 310 315 320
 Arg Phe Leu Gly Gln Glu Ile Ile Ile Glu Ser Gly Gly Thr Phe His
 325 330 335
 Ile Thr Glu Ser Pro Glu Val Ala Ala Ser Asn Gly Val Val Pro Thr
 340 345 350
 Pro Lys Ile Ile Val Glu Gln Ile Val Lys Asp Thr Leu Thr Pro Leu
 355 360 365
 Thr Glu Gly Lys Lys Phe Asn Glu Leu Cys Asn Leu Lys Ile Ala Asp
 370 375 380
 Ile Cys Cys Gly Ser Gly Thr Phe Leu Ile Ser Ser Tyr Asp Phe Leu
 385 390 395 400
 Val Glu Lys Val Met Glu Lys Ile Ile Glu Glu Asn Ile Asp Asp Ser
 405 410 415
 Asp Leu Val Tyr Glu Thr Glu Glu Gly Leu Ile Leu Thr Leu Lys Ala
 420 425 430
 Lys Arg Asn Ile Leu Glu Asn Asn Leu Phe Gly Val Asp Val Asn Pro
 435 440 445
 Tyr Ala Val Glu Val Ala Glu Phe Ser Leu Leu Leu Lys Leu Leu Glu
 450 455 460
 Gly Glu Asn Glu Ala Ser Val Asn Asn Phe Ile His Glu His Glu Asp
 465 470 475 480
 Lys Ile Leu Pro Asp Leu Thr Ser Ile Ile Lys Cys Gly Asn Ser Leu
 485 490 495
 Val Asp Asn Lys Phe Phe Glu Phe Met Pro Glu Ser Leu Glu Asp Asp
 500 505 510
 Glu Ile Leu Phe Lys Ala Asn Pro Phe Glu Trp Glu Glu Glu Phe Pro
 515 520 525
 Asp Ile Met Ala Asn Gly Gly Phe Asp Ala Ile Ile Gly Asn Pro Pro
 530 535 540
 Tyr Val Arg Ile Gln Asn Met Lys Lys Tyr Ser Pro Glu Glu Ile Glu
 545 550 555 560
 Tyr Tyr Gln Ser Lys Asp Ser Glu Tyr Thr Val Ala Lys Lys Glu Thr
 565 570 575
 Val Asp Lys Tyr Phe Leu Phe Ile Glu Arg Ala Leu Ile Leu Leu Asn
 580 585 590
 Pro Thr Gly Leu Leu Gly Tyr Ile Ile Pro His Lys Phe Phe Ile Thr
 595 600 605
 Lys Gly Gly Lys Glu Leu Arg Lys Phe Ile Ala Glu Lys His Gln Ile
 610 615 620

neb-183-cip.txt

Ser Lys Ile Ile Asn Phe Gly Val Thr Gln Val Phe Pro Gly Arg Ala
 625 630 635 640
 Thr Tyr Thr Ala Ile Leu Ile Ile Gln Ala Asn Lys Met Ala Gln Phe
 645 650 655
 Lys Tyr Lys Lys Val Ser Asn Ile Ser Ala Glu Thr Leu Asp Ser Glu
 660 665 670
 Glu Asn Thr Cys Val Tyr Ser Ser Glu Lys Tyr Asn Ser Asp Pro Trp
 675 680 685
 Ile Phe Leu Ser Pro Glu Thr Glu Ala Val Phe Thr Lys Phe Thr Glu
 690 695 700
 Ala Gln Phe Glu Lys Leu Gly Glu Ile Thr Asp Ile Ser Val Gly Leu
 705 710 715 720
 Gln Thr Ser Ala Asp Lys Ile Tyr Ile Phe Ile Pro Glu Asn Glu Thr
 725 730 735
 Ser Asp Thr Tyr Ile Phe Asn Tyr Lys Gly Lys Arg Tyr Glu Ile Glu
 740 745 750
 Lys Ser Ile Cys Cys Pro Ala Ile Tyr Asp Leu Ser Phe Gly Ser Phe
 755 760 765
 Glu Ser Ile Gln Gly Asn Ala Gln Met Ile Phe Pro Tyr Glu Ile Arg
 770 775 780
 Asp Glu Glu Ala Tyr Leu Leu Glu Glu Glu Thr Leu Glu Asn Asp Tyr
 785 790 795 800
 Pro Leu Ala Trp Asn Tyr Leu Asn Glu Phe Lys Glu Ala Leu Glu Lys
 805 810 815
 Arg Ser Leu Gln Gly Arg Asn Pro Lys Trp Tyr Gln Tyr Gly Arg Ser
 820 825 830
 Gln Ser Leu Ser Lys Phe His Asp Lys Glu Lys Leu Ile Trp Thr Val
 835 840 845
 Leu Ala Thr Lys Pro Pro Tyr Val Leu Asp Arg Asn Asn Leu Leu Phe
 850 855 860
 Thr Gly Gly Gly Asn Gly Pro Tyr Tyr Gly Leu Ile Asn Gln Ser Ile
 865 870 875 880
 Tyr Ser Leu His Tyr Phe Leu Gly Ile Leu Ser His Pro Val Ile Glu
 885 890 895
 Ser Met Val Lys Ala Arg Ala Ser Glu Phe Arg Gly Ser Tyr Tyr Ser
 900 905 910
 His Gly Lys Gln Phe Ile Glu Lys Ile Pro Ile Arg Lys Ile Asp Phe
 915 920 925
 Asp Asp Gln Asp Glu Val Asp Lys Tyr Asn Thr Val Val Thr Thr Val
 930 935 940
 Glu Lys Leu Ile Ile Thr Thr Asp Arg Ile Lys Ser Glu Ser Asn Gly
 945 950 955 960

neb-183-cip.txt

Pro Arg Arg Arg Met Leu Arg Arg Arg Leu Asp Ala Leu Ser Asn Gln
 965 970 975
 Leu Ile Gln Val Ile Asn Glu Leu Tyr Asn Ile Ser Asp Glu Glu Tyr
 980 985 990
 Thr Thr Val Leu Asn Asp Glu Met Leu Thr Ala Ala Leu Gly Glu Glu
 995 1000 1005

Lys

<210> 5
 <211> 24
 <212> DNA
 <213> Bacillus pumilus

<400> 5
 gtggaaacgg accgtattat gggt 24

<210> 6
 <211> 24
 <212> DNA
 <213> Bacillus pumilus

<400> 6
 caccagtaaa taacagggtta ttcc 24

<210> 7
 <211> 27
 <212> DNA
 <213> Bacillus pumilus

<400> 7
 ttcgtagcaa gtacgggtcca tatcagt 27

<210> 8
 <211> 27
 <212> DNA
 <213> Bacillus pumilus

<400> 8
 ccgtatgtac ttgataggaa taacctg 27

<210> 9
 <211> 24
 <212> DNA
 <213> Bacillus pumilus

<400> 9
 aggaactaag aaagttcata gctg 24

<210> 10
 <211> 24
 <212> DNA

<213> Bacillus pumilus

<400> 10
atgcggtatt atataaccca acag 24

<210> 11
<211> 24
<212> DNA
<213> Bacillus pumilus

<400> 11
tgacgtcctc ttcacctaatt tcgg 24

<210> 12
<211> 24
<212> DNA
<213> Bacillus pumilus

<400> 12
gagtttgtga agatagaacc attg 24

<210> 13
<211> 48
<212> DNA
<213> Bacillus pumilus

<400> 13
agcggatccg gaggtaaata aatgaatcaa ttaattgaaa atgttaat 48

<210> 14
<211> 42
<212> DNA
<213> Bacillus pumilus

<400> 14
aagggggcat gcttatactt atttcttcgt tctattgttt ct 42

<210> 15
<211> 51
<212> DNA
<213> Bacillus pumilus

<400> 15
caaggatccg gaggtaaata aatgcatata agtgagttag tagataaata c 51

<210> 16
<211> 36
<212> DNA
<213> Bacillus pumilus

<400> 16
ttaggatcct catttttctt ctcctaacgc cgctgt 36

neb-183-cip.txt

<210> 17
 <211> 54
 <212> DNA
 <213> Bacillus pumilus

 <400> 17
 caccaatcta gaggaggtaa ataaatgcat ataagtgagt tagtagataa atac 54

 <210> 18
 <211> 42
 <212> DNA
 <213> Bacillus pumilus

 <400> 18
 tgaaatctcg agttatcctg atccacaaca tatatctgct at 42

 <210> 19
 <211> 54
 <212> DNA
 <213> unknown

 <220>
 <223> Synthetic primer

 <400> 19
 caccaatcta gaggaggtaa ataaatgcat ataagtgagt tagtagataa atac 54

 <210> 20
 <211> 39
 <212> DNA
 <213> unknown

 <220>
 <223> Synthetic primer

 <400> 20
 gtttatacga agtgataag ctggatTTTT ctttgtctc 39

 <210> 21
 <211> 39
 <212> DNA
 <213> unknown

 <220>
 <223> Synthetic primer

 <400> 21
 gagacaaaga aaaatccagc ttatacactt cgtataaac 39

 <210> 22
 <211> 36
 <212> DNA
 <213> unknown

 <220>
 <223> Synthetic primer

 <400> 22

ttaggatcct catttttctt ctcctaacgc cgctgt 36

<210> 23
<211> 54
<212> DNA
<213> unknown

<220>
<223> Synthetic primer

<400> 23
ggtggttcta gaggaggtaa ataaatgtct aatgaaaatt ataacattga tttc 54

<210> 24
<211> 39
<212> DNA
<213> unknown

<220>
<223> Synthetic primer

<400> 24
ggtggtgagc tcctattgac ataatcgatc atcaagaag 39

<210> 25
<211> 42
<212> DNA
<213> unknown

<220>
<223> Synthetic primer

<400> 25
atagggtgga ttgcctaata ttacatcaaa gccaccattt gc 42

<210> 26
<211> 40
<212> DNA
<213> unknown

<220>
<223> Synthetic primer

<400> 26
tttgatgtaa tattaggcaa tccaccctat ataagaattc 40